

## JOVE Program Final Report

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## **I. Research**

### **Results**

Coastal reef degradation and widespread bleaching of corals, i.e. loss of pigments and/or symbiotic zooxanthellae, is increasing globally. Remote sensing has great potential for assessing the extent of reef change. However, measuring reef change from remote platforms (boats, aircraft or satellites), requires ground-truth spectral algorithms characteristic of healthy and degraded reef populations. Our laboratory experiments demonstrated, for the first time, that healthy coral colonies emit characteristic fluorescence spectra in response to excitation with short wavelength (blue or ultraviolet) light. Furthermore, following stress, e.g. increased temperature, corals lose symbiotic algae and their health is compromised. We measured changes in coral fluorescence spectra along with pigment loss in response to temperature-induced stress.

Following laboratory demonstration, we tested this approach in the field. The NASA P3 aircraft with the airborne oceanographic LIDAR system was used to map transects of coral reef in Hawaii. Ground truth samples of coral; were collected and analyzed for pigment density (pigments/surface area). Fluorescence spectra along transects showed consistent chlorophyll fluorescence peaks at 685 nm from the chlorophyll of the zooxanthellae and peaks between 400 and 570 nm believed to emanate from the coral tissue. Our results suggest that remote sensing of laser-induced fluorescence represents an efficient and effective approach to monitoring the health of coral reefs.

### **Communication with NASA Colleagues**

This project was initiated by a 10 week collaborative internship at the Goddard Space Flight Center, Observational Science Branch, Wallops Flight Facility. Dr. Frank Hoge along with Jim Yungel were my primary contacts and mentor throughout this project. This relationship proved extremely beneficial. The combination of my marine biological background with the physics and optical engineering expertise of my NASA colleagues led to a true scientific synergism. I really believe that this cross-disciplinary approach led to a significant advance, i.e. development of an entire new approach to remote sensing of coral health. My NASA colleagues were extremely helpful throughout the project. Following the 10 week on site training research experience our communications continued and led to a joint field project in remote sensing using NASA aircraft and eventually to joint presentations and publications.



#### Refereed Journal Articles Published:

Hardy, J.T., F.E. Hoge, J.K. Yungel and R.E. Dodge. 1992. Remote detection of temperature-induced coral "bleaching" using pulsed-laser fluorescence spectroscopy. *Mar. Ecol. Prog. Ser.* 88: 247-255.

\*Myers, M., J.T. Hardy, C. Mazel and P. Dustan. 1997. Optical spectra and pigmentation of Caribbean reef corals and macroalgae. Submitted. *Coral Reefs*.

#### Other Publications:

Cottone, M. 1995. Coral pigments: quantification using HPLC and detection by remote sensing. Huxley College of Environmental Studies, Western Washington University. 95 pp.

\*Myers, M. 1995. Pigmentation and fluorescence of Caribbean reef corals and macroalgae. Center for Environmental Science, Western Washington University. 59 pp.

\*Hardy, J.T. 1997. Field validation of laser-induced fluorescence spectroscopy. Proceedings of the Coral Monitoring Methodologies Workshop. Sponsored by: NOAA Coastal Services Center, Charleston, SC. Miami, Florida, September 17-18, 1996.

#### Oral and Poster Papers Presented:

Cottone, M.C. and J.T. Hardy. 1992. Remote sensing of coral reef bleaching. National Conference on Undergraduate Research, Salt Lake City, March 25-27.

Hardy, J.T. 1992. Coral reefs, climate change, and remote sensing. BFR/Sigma-Xi. Western Washington University.

\*Hardy, J.T. 1996. Spectral signatures of reefs. Coral Monitoring Methodologies Workshop. Sponsored by: NOAA Coastal Services Center, Charleston, SC. Miami, Florida, September 17-18.

\*Hardy, J.T., P. Dustan, and C. H. Mazel. 1996. Bio-optics of reef organisms in relation to remote sensing. 8th International Coral Reef Symposium, Panama, June 24-29.

\*Hardy, J.T., P. Dustan, and C. Mazel. 1995. Spectral properties of reef organisms related to remote sensing. Meeting, International Society for Reef Studies. University of Newcastle, Newcastle upon Tyne, UK, September 5-9.



Hardy, J.T. M. Cottone, M. Myers, F. Hoge, J. Yungel. 1994. Remote sensing of coral reefs using pulsed-laser fluorescence spectroscopy. Annual Meeting. American Society of Limnology and Oceanography, Miami, Florida. June 12-16.

Hardy, J.T. 1994. Coral fluorescence and remote sensing. Workshop on Remote Sensing of Coral Reefs. NOAA, Office of Global Programs. New England Aquarium, Boston. May 17-19.

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\* (Not directly supported by JOVE, but an extension of earlier JOVE research support).

Proposals Awarded (the following funded research was facilitated by initial studies completed under the JOVE Program):

Title: Quantifying coral reef pigmentation by fluorescence spectroscopy.

Authors: J.T. Hardy and P. Dustan

Agency: NOAA, Global Change Program

Funding: \$154,000

Period: Sept 93 to Aug 95

Title: Response of tropical marine symbioses to environmental stresses: an integrated approach.

Authors: G. Muller-Parker, S. Trom, J. Hardy

Agency: National Science Foundation (NSF DBI-9711075)

Funding: \$520,249

Period: June 1997-May 2000

## **II. Student Involvement and Curriculum Development**

Several students have been directly involved in this project as evidenced by the reports listed above. During the period fall 1991 to 1993 enrollment at Western Washington University, grew from 254 to 311 Environmental Science Majors. New courses based on large scale and satellite information such as *Introduction to Remote Sensing* (ENVR 442) and *Geographic Information Systems* (GEOG 358) were added to the curriculum. One course, in particular, *The Effects of Global Climate Change* (ENVR 492/592) benefited from JOVE Program information. Enrollment in this course grew from about 8 to 45.





## **ABSTRACT**

Cottone, M.C. and J.T. Hardy. 1992. Remote sensing of coral reef bleaching. National Conference on Undergraduate Research, Salt Lake City, March 25-27.

Despite their biological and economic importance, coral reefs are increasingly threatened by human activities. Recently, "bleaching" of reefs, i.e. loss of photosynthetic pigmentation, has occurred at numerous globally-distributed sites. It has been suggested that the widespread occurrence of coral bleaching represents an early warning signal of global greenhouse warming. Regardless of the cause, the extent of coral bleaching, both regionally and globally, needs to be documented and monitored. We conducted a pilot field study to evaluate the potential of using remotely-sensed laser-induced fluorescence to monitor the pigmentation of corals. The NASA P-3 research aircraft flew transects across a segment of coral reef on the northwest shore of the island of Hawaii. A pulsed laser aboard the aircraft was used to measure chlorophyll fluorescence along the reef transect. Ground truth samples of coral were collected along the transect and analyzed in the laboratory for pigmentation. Further field validation will be necessary, but this approach may make it possible to distinguish coral reef bleaching over large geographical areas with sensitivity and rapidity.

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# Remote detection of coral 'bleaching' using pulsed-laser fluorescence spectroscopy

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**ABSTRACT:** Despite their biological and economic importance coral reefs are increasingly threatened by human activities. Recently, 'bleaching' of reefs, i.e. loss of photosynthetic pigmentation, has occurred at numerous globally-distributed sites. A number of environmental stressors, including increased water temperature, can induce bleaching. Several investigators have suggested that the widespread occurrence of coral bleaching represents an early warning signal of global greenhouse warming. Regardless of the cause, the extent of coral bleaching, both regionally and globally, needs to be documented and monitored. We conducted laboratory studies to evaluate the potential of using remotely-sensed laser-induced fluorescence to monitor coral pigmentation. Five species of Caribbean corals were collected, transported to the laboratory, and maintained in aquaria. Coral samples were irradiated at either 532 nm or 337 nm with pulsed-laser light and spectral scans of fluorescence were collected at 1 nm intervals. Distinct chlorophyll fluorescence peaks at 685 and 740 nm were observed in all species examined. In corals exposed to temperature-induced stress, incipient changes in the fluorescence spectra could be detected prior to visible bleaching and coral death. Remotely-measured (aircraft or ship) laser-induced fluorescence provides a potentially useful tool for monitoring the pigmentation status of coral reefs. Field validation will be necessary, but our results suggest that it may be possible to distinguish bleaching, or even partial bleaching, over large geographical areas with sensitivity and rapidity.

## INTRODUCTION

Coral reefs are a vital global resource. They provide a rich habitat for a great diversity of marine species. As natural breakwaters, fringing and barrier reefs protect shorelines from wave-induced erosion. Numerous species utilize the sheltered waters created by reefs as nursery grounds for their young. The tourism, recreation and fishing industries, and thus the economies of many nations, depend on the presence of thriving coral reefs. Despite their biological and economic importance, coral reefs worldwide are being increasingly threatened by human activities. Recently, coral death and extensive 'bleaching' of reefs have been reported from numerous globally-distributed areas.

Symbiotic algae (zooxanthellae) within the coral polyps constitute an important source of carbon for the animal's nutrition and are essential to the health and growth of many corals (Muscantine et al. 1981, 1984).

Bleaching results from either wholesale loss of zooxanthellae from the coral tissue or from a decrease in the pigment density within individual zooxanthellae themselves (Hoegh-Guldberg & Smith 1989, Kleppel et al. 1989). A number of environmental stressors, both natural and anthropogenic, can induce bleaching (Brown & Howard 1985). These include salinity reduction, prolonged darkness, local pollution-induced stress such as dredging or deforestation with subsequent nearshore siltation, industrial and sewage discharges, non-point source storm-water runoff, and oil spills. The health of coral reefs, as with other ecosystems, is closely tied to climate. Bleaching is induced by increased water temperature and has been linked to warm water El Niño - Southern Oscillation (ENSO) events in the Pacific. A short term (several days) increase of 3 to 4 °C or a longer term increase of only 1 to 2 °C above the normal summer maximum temperature can induce bleaching (see review in Jokiel & Coles 1990).

Sublethal effects of temperature stress on corals include depressed feeding responses, reduced reproductive rates, increased extrusion of zooxanthellae and mucus, a decrease in the photosynthesis/respiration ratio (Johannes 1975), and reduced skeletal growth (Coles & Jokiel 1978, Goreau & Macfarlane 1990). Bleached corals in the Caribbean were not able to complete gametogenesis during the reproductive season following bleaching (Szmant & Gassman 1990). If stress is sublethal, corals can recover completely from bleaching and show no evidence of histological damage (Hayes & Bush 1990). However, if bleaching persists or recurs frequently, the coral will die.

Several studies have suggested that the recent world-wide occurrence of coral bleaching may represent an early warning signal of global greenhouse warming (Williams & Bunkley-Williams 1990, Hayes & Goreau 1991). In addition, corals, like most marine organisms, are very sensitive to solar ultraviolet-B radiation (Jokiel & York 1984, Hardy & Gucinski 1989). Global increases in ultraviolet radiation could adversely affect coral reefs by increasing their sensitivity to thermally-induced bleaching (Lesser et al. 1990). Recent mass bleaching of reef corals has been reported from Bermuda, Panama, the Galapagos Islands, the Caribbean, Florida Keys, Hawaii, Australia, southern Japan, Polynesia, and Indonesia. Regardless of the cause, the extent of coral bleaching, both regionally and globally, needs to be documented and monitored (D'Elia et al. 1991). As Grigg & Dollar (1990) stated, 'One of the most important limitations in assessing the effects of stress on coral reefs is the general lack of quantitative data (spatial and temporal) covering both natural and man-induced impacts.'

Fluorescence provides a non-destructive tool for monitoring changes in photosynthetic pigments. Photosynthesis involves trapping of light energy by photosynthetic systems I and II (PS-I and PS-II). Part of the trapped energy decays within 1 to 2 ns emitting photons (fluorescence). Peaks in the action spectra of *in vivo* chlorophyll fluorescence are believed to relate to different forms of chlorophyll *a* (chl *a*) and different proportions of chl *a* and chl *b* within PS-I and PS-II (Govindjee et al. 1971). The exact location of fluorescence peaks differs between species, but prominent peaks are often found for PS-II at 683 to 687 nm (with a satellite peak at 740 nm) and for PS-I at 720 nm.

The technology may now exist to rapidly assess the bleaching of coral reefs over large geographic areas. Airborne oceanographic LIDAR (light detection and ranging) systems use a pulsed-laser from an aircraft to measure the fluorescence emitted by aquatic or terrestrial chlorophyll pigments along the aircraft's flight path. In the last decade ecological applications of airborne laser-induced fluorescence (ALIF) have greatly

expanded. Biological studies have included quantification of offshore marine phytoplankton (Hoge & Swift 1981, 1983, 1985), mapping of coastal and terrestrial vegetation (Hoge & Swift 1983, Hoge et al. 1983) and assessment of environmental stress on terrestrial plant communities (Chappelle et al. 1984, Schmuck & Lichtenthaler 1986). Zooxanthellae within the tissues of coral also exhibit pigment fluorescence when irradiated at wavelengths of 532 nm or less. In addition, many corals contain granules within the polyp tissues (separate from zooxanthellae) which fluoresce when irradiated with ultraviolet wavelengths (Mazel 1988, Logan et al. 1990).

We describe here laboratory results which suggest that remote (boat or aircraft platform) laser-induced fluorescence has great potential as a useful tool for monitoring and rapidly assessing the pigmentation of coral reefs over large regions.

## METHODS

Individual coral colonies were collected between September 30 and October 16, 1991 from depths of 7 to 10 m near Fort Lauderdale, Florida, USA. Corals were transported live to the NASA, Goddard Space Flight Center, Wallops Flight Facility, Virginia, USA, using the dry perlite method (Bronikowski 1982, Logan et al. 1990). Within 24 h of collection corals were placed in 38 to 75 l re-circulating seawater aquaria and maintained at a temperature of 27 °C and a salinity of 30 ppt. The aquaria were continuously illuminated with cool-white fluorescent lamps at an intensity of about 100 µW (spectrally-integrated from 400 to 750 nm, measured with a United Detector Technology Model 40A). Water quality parameters (pH, ammonium and nitrite) were monitored daily and maintained at acceptable levels during the course of the experiments.

Within 1 to 30 d of collection fluorescence spectra were measured on samples of 7 coral species: *Agaricia agaricites*, *Dichocoenia stokesi*, *Meandrina meandrites*, *Montastrea cavernosa*, *Siderastrea siderea*, *Solenastrea bournoni*, and *Stephanocoenia michelinii*. For most species, plugs were cut from the corals using a drill press equipped with a 2 cm diameter hole saw. In the case of the more convoluted *Meandrina meandrites* a wedge, approximately 1 × 3 cm, was cut from the distal portion of the colony with a metal hack saw blade. The coral samples were immediately placed in quartz cuvettes containing seawater, irradiated with pulsed-laser light, and fluorescent emission spectra collected with a spectrofluorometer (Hoge et al. 1983) (Fig. 1). Scan times were generally about 1 min.

Two different lasers were used in separate experiments to provide excitation wavelengths comparable

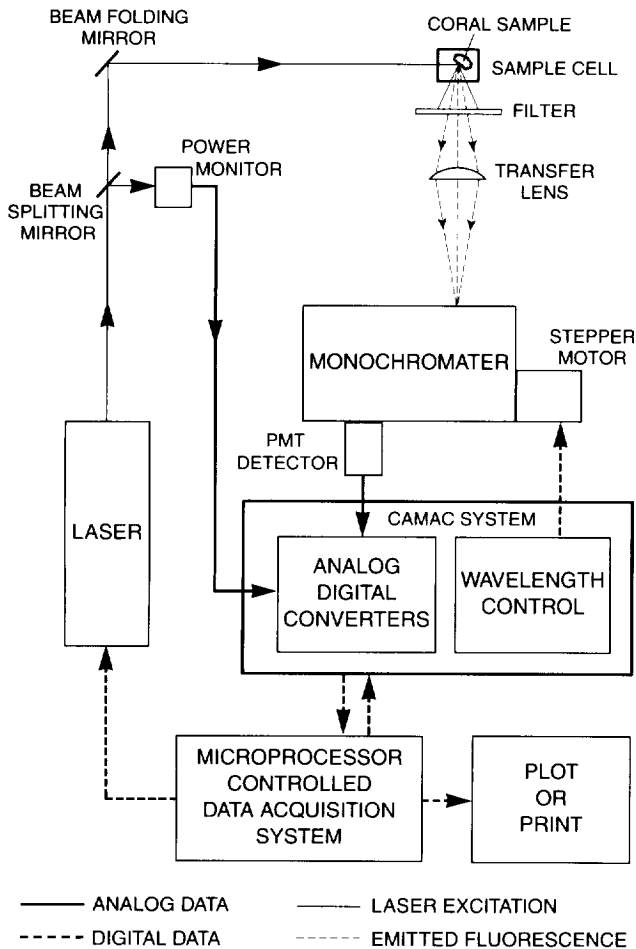


Fig. 1. Configuration of laboratory pulsed-laser fluorescence spectrophotometer

to airborne LIDAR equipment. An International Laser Systems model NT274 frequency doubled Nd:YAG laser provided approximately 1.0 mJ, 532 nm excitation energy, and an Avco Everett model C-5000 nitrogen laser provided approximately 0.2 mJ, 337 nm excitation energy. Both laser pulse widths were approximately 15 ns.

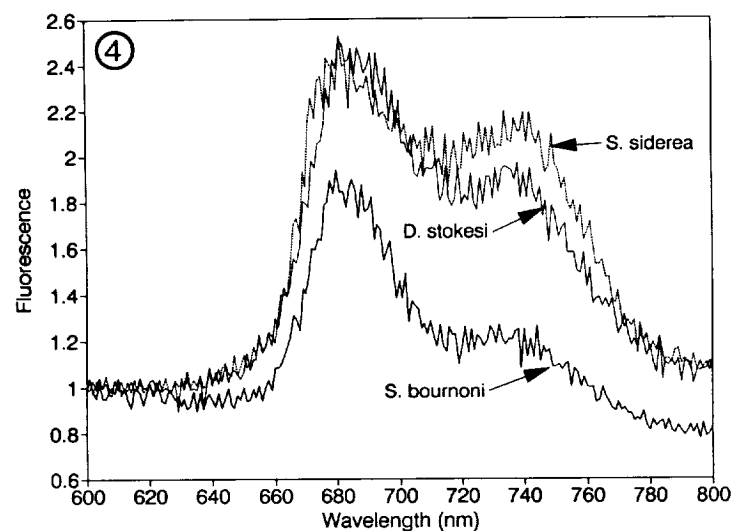
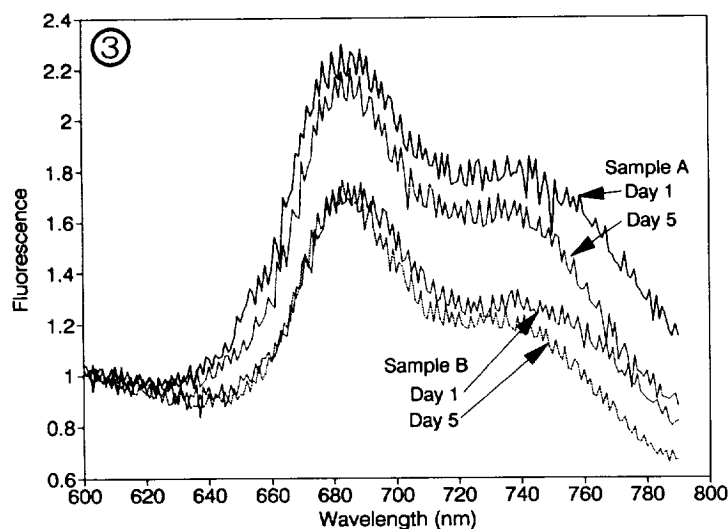
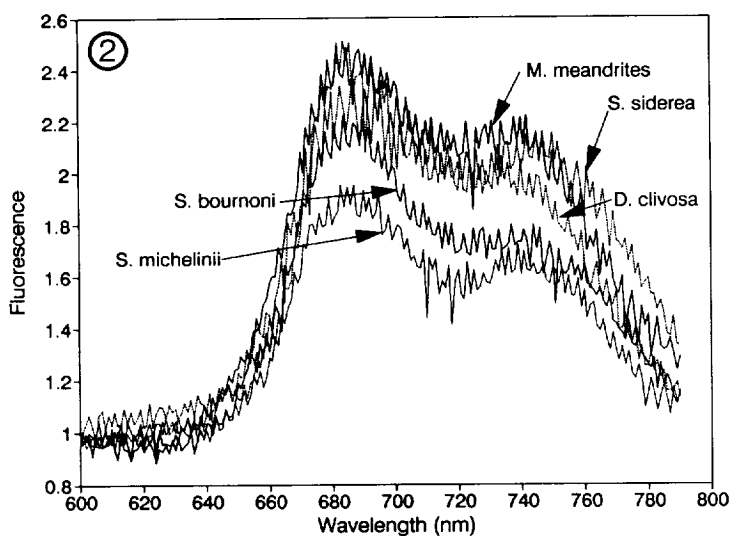
The laser radiation was directed into quartz cells holding the coral sample immersed in seawater. The resulting fluorescence was viewed normal to the incidence angle of the exciting laser radiation by an  $f/3$  collection and transfer lens which provided optical matching to the Instruments SA model H-10 0.1 m Ebert configuration monochromator. In order to reject the strong scattering of laser radiation from the coral samples, longpass filters were placed between the sample and transfer lens. Filters consisted of a Schott Glass OG-550 for  $<550$  nm (Nd:YAG laser), or a 2,7-dimethyl-3,6-diazacyclohepta-1,6-diene perchlorate liquid filter to reject radiation  $<350$  nm (nitrogen laser).

The grating of the monochromator was driven by a stepper motor through the CAMAC crate bus providing data system control of the grating in 0.5 nm steps. The output radiation from the monochromator was measured by a Hamamatsu R-666S photomultiplier tube (PMT), whose gain was controlled by an adjustable high voltage power supply. The output of the PMT was delayed to allow the gates triggered by the laser pulse to be generated, so that the gate pulse arrived simultaneously with the PMT output pulse, at the LeCroy model 2249SG charge digitizer. The gate pulse generated is approximately 50 ns wide allowing integration of resulting PMT output by the charge digitizer. The digital information from the charge digitizer is interfaced over the CAMAC crate data bus to the data collection system. The data collection software controls the grating steps and digitizer reading, allowing averaging of 30 pulses (nm step)<sup>-1</sup>. Fluorescence spectral scans were collected at 1 nm intervals from 500 to 800 nm (Nd:YAG laser) or 300 to 800 nm (nitrogen laser). The resultant digital information was written to an ASCII data file, and plotted and analyzed using a standard MS-DOS spreadsheet program. Fluorescence values at all wavelengths were normalized to that at 600 nm.

The effects of temperature-induced 'bleaching' on the fluorescence spectra within the same and among different coral species were examined. We maintained cuvettes, containing the coral samples, in aerated temperature-controlled seawater at 35 °C under approximately the same illumination conditions as the aquaria (above) for 5 d and removed the cuvettes daily for spectral scans. Expelled pigments were rinsed from the cuvettes with clean seawater prior to performing scans. Duplicate cores of *Solenastrea bournoni* were maintained at 27 °C as a control. Laser scans were also taken following an acute temperature exposure experiment. Samples of *S. bournoni* were exposed to a 35 °C mean temperature for 4 h including a brief elevation to 40 °C and then returned to 27 °C.

We also investigated an alternative method of measuring LIF of corals. A dual-cable fiber optic probe was placed directly over the coral colony with the probe head approximately 1 cm from the polyp tissue. Laser light was channeled through an excitation fiber cable to the probe head. An emission collection fiber cable in the probe head, oriented 90° to the excitation cable, channeled the reflected light to the scanning monochromator.

Studies of marine phytoplankton (Hoge & Swift 1981, 1983, 1985) and terrestrial plants (Hoge et al. 1983, Chappelle et al. 1984, Nelson et al. 1984) have applied different algorithms to remotely sensed color reflectance or fluorescence peaks in order to quantify chlorophyll pigments. For example, a curvature



algorithm was effectively used for remote active-passive (laser-induced) correlation spectroscopy (Hoge & Swift 1986). Here we apply a modified curvature algorithm to quantify LIF of corals, combining information contained in both the 685 nm and 740 nm peaks.

$$C = (F_{685})^2 / (F_{715})(F_{655}) + (F_{740})^2 / (F_{770})(F_{710}) \quad (1)$$

where  $C$  = relative strength of chlorophyll fluorescence;  $F_i$  = fluorescence at wavelength  $i$ , normalized to 600 nm.

Photosynthetic pigments were measured in samples of *Solenastrea bournoui* exposed to temperature stress. At 0, 24 and 48 h of exposure to 35 °C, duplicate 2 cm diameter plugs were removed from the colony and ground with a mortar and pestle in 90 % acetone. Spectral absorbance was measured using a Perkin Elmer Lambda 3B UV/Vis spectrophotometer following the procedures of Parsons et al. (1988). Pigment concentrations were expressed per unit surface area of coral.

## RESULTS

### General similarity of species spectra

All coral species, irradiated with a Nd:YAG laser at 532 nm, demonstrated a remarkable similarity in fluorescence spectra (FS) (Fig. 2). In most species examined chlorophyll fluorescence peaks occurred consistently at about 685 and 740 nm. FS of different samples of the same species differed in amplitude, probably due to different aspect angles and reflectance, and possibly different densities of zooxanthellae, but the shape of the FS did not differ between samples. FS decreased only slightly on cut samples removed from the colony and retained in cuvettes in an aquarium at 27 °C for up to 5 d (Fig. 3).

Fig. 2. Fluorescence spectra of unstressed coral species, irradiated at 532 nm. All 5 species show peaks at 685 and 740 nm

Fig. 3. *Solenastrea bournoui*. When plugs were removed from 2 different colonies (A & B) of coral and maintained at 27 °C, the fluorescence spectrum degraded only slightly after 5 d

Fig. 4. Fluorescence spectra of 3 coral species irradiated at 532 nm using a dual fiber optic probe

The fiber-optic probe placed, non-destructively, directly over the live coral colony proved equally effective at recording FS of corals (Fig. 4). The normal 685 nm peak was shifted toward 680 nm, perhaps as a result of transmission through the optic fibers. Variations in the fluorescence strength over the surface of the coral could be 'mapped' as the probe was moved from one area of the colony to another.

The endolithic alga (perhaps a blue-green alga) within the skeletons of *Montastrea cavernosa* and *Agaricea agaricites* was also examined. When samples from inside (1 cm or deeper) the skeleton were irradiated the alga emitted a spectrum quite different from that of the intact live coral on the surface of the colony. The LIF spectra showed a broad peak with a maximum between 720 and 730 nm (Fig. 5).

*Solenastrea bournoni*, irradiated with the nitrogen laser at 337 nm, demonstrated a broad fluorescence band from about 350 to 550 nm along with the typical chlorophyll fluorescence peaks at 685 and 740 nm (Fig. 6). The apparent peak at 495 nm is due to uncorrected blaze angle inefficiencies in the reflection grating of the monochromator.

### Effects of temperature stress

When *Solenastrea bournoni* was subjected to an acute temperature stress of 35 to 40 °C over a 4 h period and then returned to 27 °C, the amplitude of the FS decreased over the following 6 d period (Fig. 7). Likewise, when other species were held constantly at 35 °C, the FS decreased over a 5 d period as the coral 'bleached' and eventually died (Fig. 8a, b, c).

Although peak heights of fluorescence differed somewhat between coral species, the

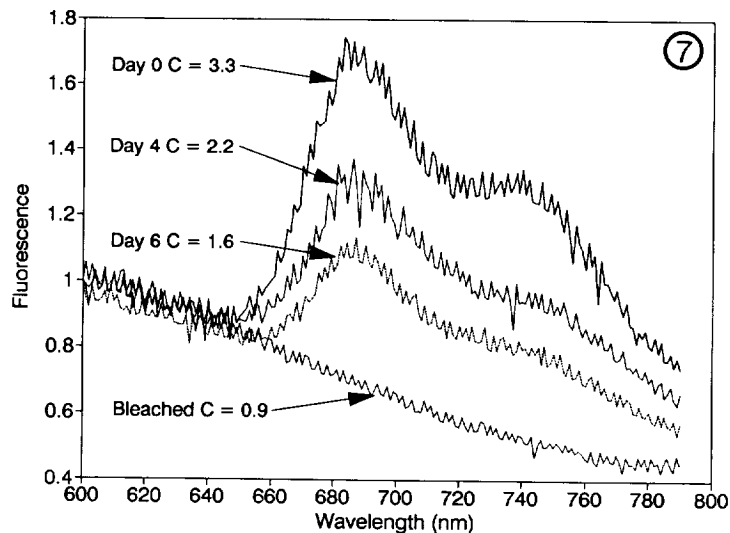
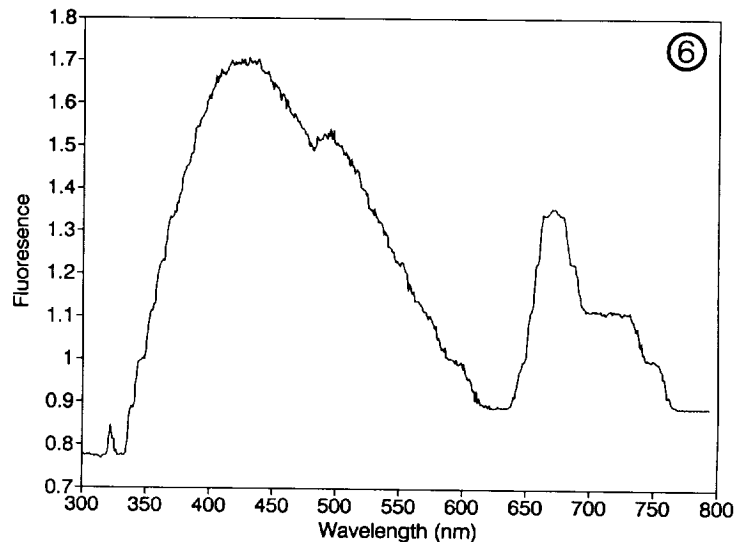
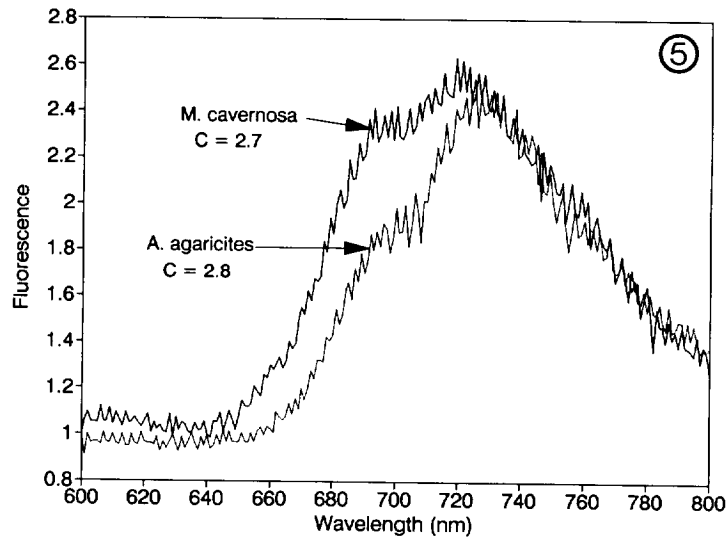
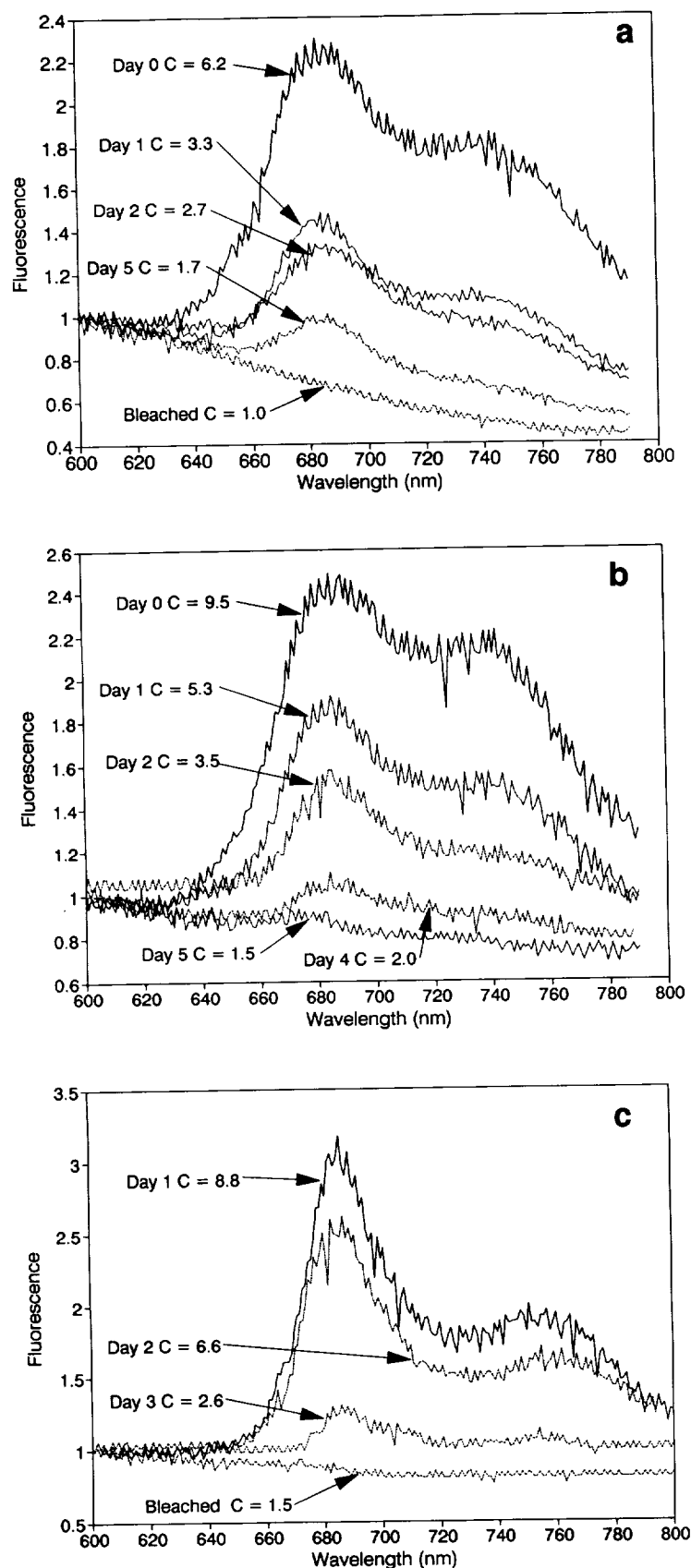


Fig. 5. Fluorescence spectra of endolithic alga irradiated at 532 nm

Fig. 6. *Solenastrea bournoni*, irradiated with a nitrogen laser at 337 nm, showed a broad fluorescence peak centered around 425 nm and chlorophyll peaks between 660 and 750 nm

Fig. 7. *Solenastrea bournoni*. Fluorescence spectrum of coral declines with time following acute temperature stress. C: computed curvature algorithm



curvature algorithm  $C$  varied by only  $\pm 1.6\%$  between species (Fig. 2).  $C$  also served as a sensitive indicator of the decline in the chlorophyll fluorescence. In normal corals  $C$  was generally  $>6$ . In temperature-stressed coral  $C$  declined over a period of 5 d to values of about 1 or less (Fig. 8a, b, c).

Extracted pigments of *Solenastrea bournoni* showed distinct chlorophyll (665 nm) and carotenoid (480 nm) absorption peaks which decreased after exposure of the coral to elevated temperature ( $35^{\circ}\text{C}$ ) stress (Fig. 9). During 48 h of temperature stress the quantity of chl *a*, *b*, *c* and carotenoids declined by 55, 47, 87, and 54 %, respectively (Fig. 10).

## DISCUSSION

To our knowledge, these results represent the first recorded spectral scans of active laser-induced fluorescence in corals. Water column profiles of laser-induced phytoplankton pigment fluorescence spectra have been collected using a shipboard argon laser connected to a fiber-optic probe (Cowles et al. 1989, 1990). Passive (solar-induced) fluorescence has been measured in corals using a single band-pass filter (683 nm centered) fluorometer (Morrow et al. 1991). However, passive single-band fluorescence provides only a crude measure of coral pigment composition. Our approach, using scans over a wide spectral range, promises greatly increased sensitivity. The computation of curvature algorithms for different peaks should allow detection of subtle changes in coral pigments induced by environmental stress.

Bleached coral samples from the field have been found to contain greatly reduced quantities of photosynthetic pigments (Kleppel et al. 1989). In Florida, chl *c*, peridinin, and diadinoxanthin levels were 35, 17, and 20 times higher in normal corals than in bleached ones (Kleppel et al. 1989). In our experiments, before bleaching, the density of

Fig. 8. Fluorescence spectra of (a) *Solenastrea bournoni*, (b) *Meandrina meandrites* and (c) *Siderastrea siderea* declines during continuous temperature stress ( $35^{\circ}\text{C}$ ).  $C$ : computed curvature algorithm



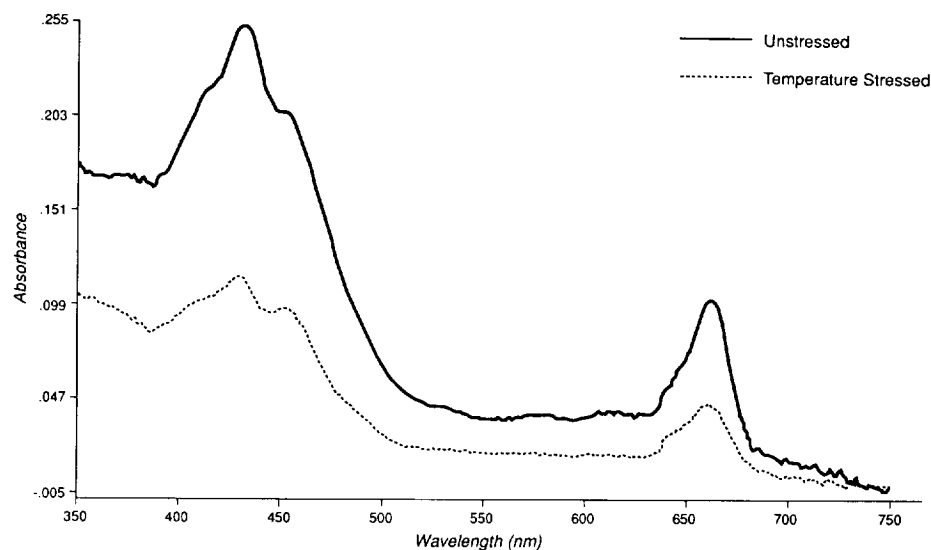


Fig. 9. *Solenastrea bournoni*. Absorption spectra of acetone extract of pigments of temperature-stressed and unstressed coral

pigment ( $\mu\text{g cm}^{-2}$ ) over the surface of *Solenastrea bournoni* was about half that reported for *Montastrea annularis* from Florida (Kleppel et al. 1989). During our temperature-induced stress experiments both LIF and pigment concentrations in *S. bournoni* declined.

Our results show clear and dramatic changes in active fluorescence spectra of corals due to temperature stress. The mean temperature maximum over the past 50 yr at Miami Beach, Florida, is about 31 °C. In this study, exposure of corals to a temperature 4 °C greater than this summer maximum resulted in bleaching and death. Our results do not differentiate clearly whether the pigment loss was due to chlorophyll degradation or to expulsion of zooxanthellae. However, masses of pigment sug-

gestive of expelled zooxanthellae were visible in the stressed samples. Further study is needed (possibly using cultured zooxanthellae) to examine possible changes in pigmentation and FS resulting from chronic exposure to smaller temperature elevations.

Can LIF measurements from remote platforms (aircraft or boats) be used to measure the pigmentation of coral reefs? Our laboratory results indicate strong fluorescence signals when corals are irradiated at 337 or 532 nm. The NASA airborne laser (Hoge 1986) can penetrate to 30 m in clear oceanic waters. Phytoplankton pigments in the water column pose a potential source of interference. However, phytoplankton chl *a* densities in tropical waters are often less than 0.5  $\text{mg m}^{-3}$  (Behrenfeld et al. 1992). In a 10 m water column this is equivalent to less than 0.5  $\mu\text{g chl a cm}^{-2}$ . Chl *a* densities of coral colonies are likely to be about 3  $\mu\text{g cm}^{-2}$  (this study) to 6  $\mu\text{g cm}^{-2}$  (Kleppel et al. 1989). It is likely, therefore, that the high pigment densities of corals would produce a 680 nm fluorescence signal significantly stronger than the overlying phytoplankton, at least at depths down to 10 m. In any case, a nano-second pulsed LIDAR system will detect 2 signals, the first from the water surface and the second from the bottom. The instrument can be time (depth) 'gated' to detect fluorescence only from the bottom (coral) surface and to ignore the water column (phytoplankton) signal.

Overflights of airborne LIF sensors offer the potential advantage of covering large areas rapidly, but pose a problem of scale and resolution. At an overflight speed of 100  $\text{m s}^{-1}$  and 20 pulses (samples)  $\text{s}^{-1}$ , 1 fluorescence measurement (sample) would be collected about every 5 m. Several overflights might be necessary to adequately characterize the pigmen-

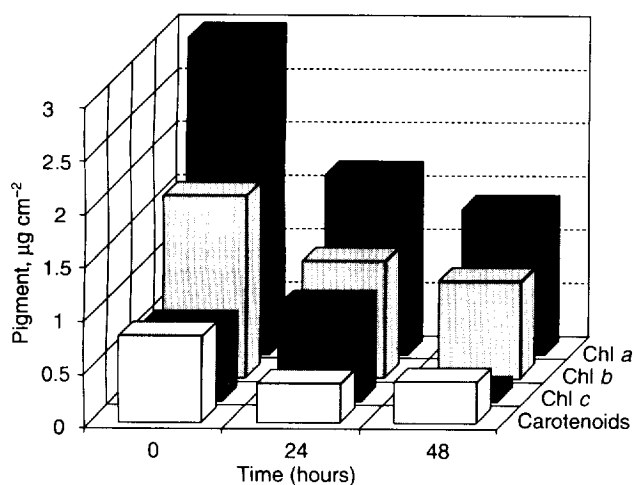


Fig. 10. *Solenastrea bournoni*. Decrease in photosynthetic pigments of coral exposed to elevated temperature of 35 °C for 48 h

tation of a coral reef. Also, the longer-wavelength 740 nm fluorescence signal could be significantly attenuated by the water column.

Such difficulties could be overcome by the use of the fiber optic probe technique demonstrated in this study. This probe could allow selective assessment of individual corals using SCUBA as well as scanning from boats along transects at a fixed distance from the bottom.

We have reported on fluorescence of chlorophyll pigments within the zooxanthellae of corals. Many coral species also exhibit fluorescence when irradiated with ultraviolet light *in vivo*. Such UV fluorescence is the product of small granules in the epidermis or gastrodermis of the polyp animal itself (Mazel 1988, Logan et al. 1990). It has been hypothesized that fluorescence from these granules may act as a filter for harmful solar ultraviolet, converting it to longer wavelengths useful for photosynthesis by the zooxanthellae (Kawaguti 1969).

The broad-band fluorescence peak from 350 to 550 nm, resulting from 337 nm excitation (nitrogen laser), may result from the accessory pigments carotene peridinin, dinoxanthin and diadinoxanthin known to be present in zooxanthellae (Taylor 1967). Further examination of this band could clarify its origin and usefulness as an environmental indicator.

Our laboratory results suggest that remote (ship or aircraft) active laser systems represent potentially powerful tools for coral reef research. Systems such as the NASA AOL would allow remote detection of pulsed laser-induced pigment fluorescence from coral, along with simultaneous measurement of passive solar-induced upwelled radiance, sea surface temperature and very accurate bathymetry. The acquisition of airborne upwelled radiances should allow more optimum selection of appropriate satellite bands and development of attendant algorithms. It should be possible to distinguish areas of coral reef bleaching, or even partial bleaching, with greater sensitivity than direct visual observation. In addition, of course, there is the advantage, using aircraft, of covering large areas rapidly, i.e. about 4 miles of reef per minute.

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